

Thermal response in pre-imaginal biology of *Ochlerotatus albifasciatus* from two different climatic regions

M. J. GARZÓN and N. SCHWEIGMANN

Grupo de Estudio de Mosquitos, Departamento de Ecología, Genética y Evolución, Instituto de Ecología Genética y Evolución de Buenos Aires (IEGEB)- Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

Abstract. The biological processes on mosquito could be variable in response to local climatic characteristics. The thermal effects on time and the rate larval development, immature survival and adult size in local populations of *Ochlerotatus albifasciatus* (Macquart) (Diptera: Culicidae) from cold (Sarmiento) and temperate (Buenos Aires) regions from Argentina were evaluated. This species affects livestock production and human health. Larvae of both regions were placed in breeding thermal baths (11–32 °C range). Development and survival were recorded daily until adult emergence. The development temperature threshold and thermal constant for Sarmiento (4.59 ± 3.08 °C, 204.08 ± 7.83 degree days) was lower and higher than Buenos Aires, respectively (8.06 ± 1.81 °C, 149.25 ± 2.6 degree days). At cold temperatures (11–16 °C), Sarmiento larvae demonstrated 5 days faster development and higher survival (56%) than Buenos Aires (15%), whereas at warm temperatures (20–32 °C) were up to 2 days slower and similar survival (16% vs. 18%). The size did not show differences between populations. An *Ochlerotatus albifasciatus* population seems to present local thermal responses. The favourable temperature for survival and rate of development would vary within a cold or warm range, and these differential responses would explain the wide geographical distribution in different climatic regions of southern South America.

Key words. Development threshold, development time, mosquitoes, survival, thermal constant.

Introduction

Insect biological processes depend on a specific temperature range of the environment. Above the lower threshold of this range, an increase in the temperature of the environment results in an increase in the metabolic rate of the organism (Williams & Felmate, 1992). This affects the development and growth of larvae as well as the size and fecundity of adults. In immature stages, an increase in the temperature causes a decrease in the length of each stage and, as a result, a speeding up of the development and emergence (Sweeney & Vannote, 1981). Low temperatures can produce large adults whereas high temperatures can produce small adults (Wise, 1980).

The relationship between development time (DT) and temperature has been expressed using linear approximations that have allowed estimating the lower temperature threshold for development and the thermal constant, with some margin of error (Honek, 1996; Tun-Lin *et al.*, 2000) These parameters of development tend to be used to detect restrictions on development imposed by geographical, taxonomic and allometric effects (Honek, 1999).

Among the various species of mosquitoes, *Ochlerotatus albifasciatus* (Macquart) is characterized by breeding in 'flood waters'. This species is a voracious biter feeding on both humans and domestic animals, affects negatively the livestock production (Fava *et al.*, 2001) and has shown a vectorial capacity

Correspondence: Maximiliano J. Garzón, Intendente Güiraldes 2160 – Ciudad Universitaria – C1428EGA – Buenos Aires, Argentina. Tel.: ++54 +11 4576-3354; Fax: ++54 +11 4576-3354. E-mail: maxigarzon@yahoo.com.ar

for the Western equine encephalitis (WEE) virus (Avilés *et al.*, 1992).

The northern end of *Oc. albifasciatus* distribution reaches Santa Cruz de la Sierra, Bolivia (Prosen *et al.*, 1962), whereas the southern end of its distribution reaches Tierra del Fuego, Argentina (Bachmann & Bejarano, 1960), thus covering different climatic regions. It is expected that a species with a wide latitudinal distribution that can proliferate in both warm and cold areas has developed different biological features in response to the climate of different latitudes (Trimble & Smith, 1978).

The development time of the flood water mosquitoes demonstrate an increase or decrease according to the habitat characteristics which includes biotic (density) or physical factors (temperature and habitat water level) (Schäfer & Lundström, 2006). This type of response could vary according to climatic conditions in different latitudes.

In *Oc. albifasciatus*, there are not studies about variation in the ecology and the development among populations from different latitudes.

Thus, the aim of this work was to carry out a comparative study of the effects of temperature on the time and rates of larval development, the survival of the different immature stages and the size of adults at the time of emergence, in *Oc. albifasciatus* specimens from two different climatic regions within its distribution area.

Materials and methods

Collection and preparation of biological material

Ochlerotatus albifasciatus females were collected in Buenos Aires (34°36'S, 58°26'W) and Sarmiento (45°35'S, 69°05'W) (Fig. 1). The first site is located in the Pampas region, characterized by a humid temperate climate (Peel *et al.*, 2007) with warm summers (average 25 °C) and cool winters (average 12 °C).

The second site is located in a valley of the Patagonian region, with a arid steppe climate (Peel *et al.*, 2007). The Patagonian valley represents a wet patch in a desert steppe matrix, the summers are warm with a mean 18 °C but with daily desert thermal amplitude, whereas the winters are very cold (mean 4 °C).

Females were caught during December to January (2010–2011) in Buenos Aires and January to February (2011) in Sarmiento. Once captured, using a vacuum cleaner, mosquitoes were placed in breeding boxes (30 × 32 × 31 cm³) with a wall and wooden floor, two plastic sides, a tulle ceiling and a front with a fabric sleeve to manipulate mosquitoes and containers. Females were acclimated to the room temperature of the laboratory (≈23 °C) and a photoperiod of LD 14: 10 h and fed with a solution of water with sugar by means of a covered container and in contact with absorbent paper. To maintain a high RH (relative humidity) (>80%) in the breeding boxes, a damp cloth, which was moistened whenever necessary, was placed on the base and roof of each box. Finally, each box was covered with a plastic bag to maintain these conditions.

Females were blood-fed on anaesthetized mammal (a lactating mice or a rabbit) accord to regulations for the care and use of laboratory animals at the University of Buenos Aires (R

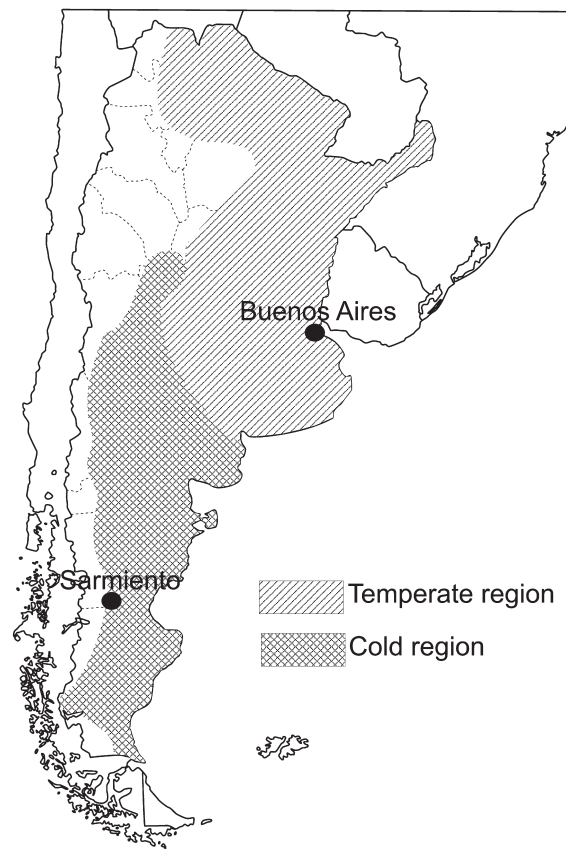


Fig. 1. Study sites and climatic regions of Argentina (by Peel *et al.*, 2007) where females of *Ochlerotatus albifasciatus* were captured.

4081/2004). The place for oviposition was a Petri dish with damp cotton, covered either with absorbent paper or a damp cloth. After oviposition, the eggs in the substrate were placed inside a small nylon bag hermetically closed. The eggs collected were left at room temperature for 8–9 days to ensure full embryonic development (Fava *et al.*, 2001) and subsequently stored (3–4 months) at approximately 5 °C to ensure one greater success of hatching (Campos, 2008) for the experiment.

To obtain first instars larvae for the experiment, the eggs were removed from cold storage conditions (5 °C) and placed at room temperature (≈23 °C) with a photoperiod of LD 14: 10 h for 2 weeks to acclimate them and reverse a possible state of diapause (Campos & Sy, 2006). Then, the eggs were placed on plastic trays (12 × 12 × 6 cm³) and flooded with tap water with 10 mL of a solution containing water and dried Brewer's yeast to stimulate hatching (Campos & Sy, 2006; Campos, 2008).

Assay

The first-instar larvae hatched within the first 14 h after flooding were individually placed in cylindrical containers measuring 3 cm in diameter and 5 cm high with 10 mL of dechlorinated tap water. Each container was randomly chosen for a specific treatment.

Treatment consisted of groups of 30–40 larvae exposed to different constant temperatures, within thermal trays (Loetti

et al., 2007). The breeding temperatures were from 11 to 32 °C (3° step). We performed from three to four sequences (2–7 simultaneous constant temperatures/sequence) up to cover approximately the entire range (11, 14, 17, 20, 23, 26–12, 15, 18, 21, 24, 27, 30–13, 16, 19, 22, 25, 28, 31–29, 32 °C).

This was performed in one population and then in the other. As the temperatures of the trays could be affected by ambient conditions, we placed temperature data loggers (Hobo®; Onset Computer Corporation, Bourne, MA, U.S.A.) in the trays, and we took the mean registered temperature instead of the system thermostat sensors reading. For analysis, we considered only those trays (temperatures) that showed water thermal variations below 0.5 °C.

The photoperiod used was LD 14 : 10 h and the larval instars were fed daily with an aliquot of dog chow (Purina®; San Luis, MO, U.S.A.) according to Gerberg *et al.* (1994) for *Aedes* instars (I: 0.2 mg/day, II: 0.3 mg/day, III: 0.4 mg/day, IV: 0.6 mg/day).

The water level in the containers was kept constant by adding a volume equivalent to that of evaporated water. To avoid the stress of the larvae in the containers, there was only maintenance carried out (cleaning, water recharge, etc.) instead of replacing the water during the experiment. The larval stage was recorded daily and dead larvae and pupae, as well as exuviae, were removed and fixed in 97% ethanol. The emerging adults were removed from the thermal tray and placed in a freezer (–12 °C) for the time necessary for them to die. The left wing of each adult was removed, photographed and measured with the help of a stereoscopic microscope and a camera (Leica® S8 APO, Leica® DFC 295, Solms, Germany), and its length measured from the apex (extreme of the R3) to the alula base, and used as an indicator of the size of the body of the adult (Clements, 1992). The software used was Leica Application Suite V3.7 (2010).

Data analysis

The total development time was calculated as the time (in days) elapsed from the hatching of the eggs until the emergence of the adults, whereas the development time of each instar was calculated as the difference between the day on which a particular instar was reached and the day on which it passed to the next. The beginning of the treatment was taken as day 0 (D=0). Each individual was considered a replicate for each thermal tray. The development time of each individual was plotted as a function of the breeding temperature for each population, and a descriptive analysis of the response was conducted. To statistically compare the response of development time vs. temperature in both populations, we decided to linearize the function (see Statistical analysis).

To estimate the lower development threshold and the thermal constant for both populations, we carried out a linear regression analysis of the development rate (reciprocal of development time = 1/DT) vs. temperature, according to Teng & Apperson (2000). The thermal range used for the estimates was 11–25 °C, because higher temperatures revealed approximately similar rates.

Overall survival for each rearing temperature was calculated as the number of emerging adults relative to the initial number

of first-instar larvae per treatment. The survival of each instar, at a given temperature, was calculated as the proportion of individuals that passed to the next instar, relative to the number of individuals that initiated the instar. To explain the differences between populations with respect to development and overall survival, we compared the times of development and survival at the instar level. To this end, we grouped the individuals according to three arbitrary thermal ranges: cold (11–16 °C), warm (17–22 °C) and hot (25–32 °C).

For each population, every adult was associated with a rearing temperature and a wing length. The length of the left wing (assuming symmetry between left and right wings) was plotted as a function of temperature and adjusted to a linear function.

Statistical analysis

The relationship between development time vs. temperature was linearized ($y = mx + c$) by transforming the data of the dependent variable to the natural logarithm [Ln (DT)] (Zar, 1996). We then estimated the confidence interval for each regression function and calculated the coefficient of determination (r^2) to evaluate the good fit of the model (Zar, 1996).

To statistically compare the regression slopes obtained, we conducted the test for parallelism or homogeneity of slopes (Chatterjee *et al.*, 2000). The non-parametric analysis Mann–Whitney *U*-test (Zar, 1996) was applied for the analysis of development time as a function of temperature for each instar. Total survival and survival of each instar were analysed with a test of proportions for independent samples (Fleiss, 1981). For the morphometric analysis, a linear regression analysis (Zar, 1996) was carried out for each population and a test for parallelism between slopes was used to compare populations (Chatterjee *et al.*, 2000).

Results

The straight lines of the linear fit of Ln (DT) vs. temperature in Buenos Aires ($r^2 = 0.91$, $m = -0.1$, $P_m < 0.0001$, $c = 4.6$, $n = 104$) and Sarmiento ($r^2 = 0.93$, $m = -0.07$, $P_m < 0.0001$, $c = 4.14$, $n = 109$) had significantly different slopes (test for parallelism, $P < 0.0001$) (Fig. 2A). At low temperatures (11–16 °C), the estimated development time of the individuals from Buenos Aires was up to 5 days slower than that of individuals from Sarmiento Valley, whereas at warmer temperatures (20–32 °C), the larvae from Buenos Aires developed up to 2 days faster (Fig. 2B). Approximately at 18 °C, both populations showed similar development times.

In both populations of *Oc. albifasciatus*, the development time of every instar showed an inverse relationship with temperature. The instars that best explained differences in total development time were instars I, IV and pupa for low temperatures; instars II, III and IV for warm temperatures and only instar I for hot temperatures (Table 1). However, in cold temperatures, the instars II from Sarmiento were slower in development than those of Buenos Aires.

The development rate as a function of temperature showed a significant ($P < 0.0001$) linear fit for Buenos Aires ($m = 0.0067$,

Table 1. Average development times \pm SD (days) per instar for Buenos Aires and Sarmiento populations to different thermal ranges.

| Instar | 11–16 °C (low) | | 17–22 °C (warm) | | 25–32 °C (hot) | |
|--------|------------------------|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| | Buenos Aires | Sarmiento | Buenos Aires | Sarmiento | Buenos Aires | Sarmiento |
| I | *3.73 \pm 1.35 (209) | 2.91 \pm 0.72 (119) | 2.14 \pm 0.46 (174) | 1.97 \pm 0.46 (38) | 1.09 \pm 0.29 (158) | *1.88 \pm 0.40 (80) |
| II | 3.56 \pm 1.43 (165) | *4.27 \pm 1.56 (105) | 1.69 \pm 0.51 (138) | *2.03 \pm 0.29 (36) | 1.22 \pm 0.42 (128) | 1.13 \pm 0.34 (68) |
| III | 4.47 \pm 1.93 (101) | 4.49 \pm 1.79 (95) | 2.24 \pm 0.91 (75) | *2.71 \pm 0.82 (31) | 1.65 \pm 0.66 (60) | 1.46 \pm 0.55 (46) |
| IV | *8.32 \pm 2.62 (59) | 7.44 \pm 2.59 (81) | 3.56 \pm 1.43 (41) | *4.08 \pm 0.88 (24) | 2.35 \pm 0.75 (33) | 2.35 \pm 0.55 (31) |
| P | *6.24 \pm 1.64 (48) | 5.48 \pm 1.20 (67) | 2.82 \pm 0.72 (34) | 2.90 \pm 0.31 (20) | 1.74 \pm 0.45 (24) | 1.587 \pm 0.51 (22) |

The sample size is indicated in brackets.

Asterisks indicate significant differences when comparing the two regions at each instar for each thermal range.

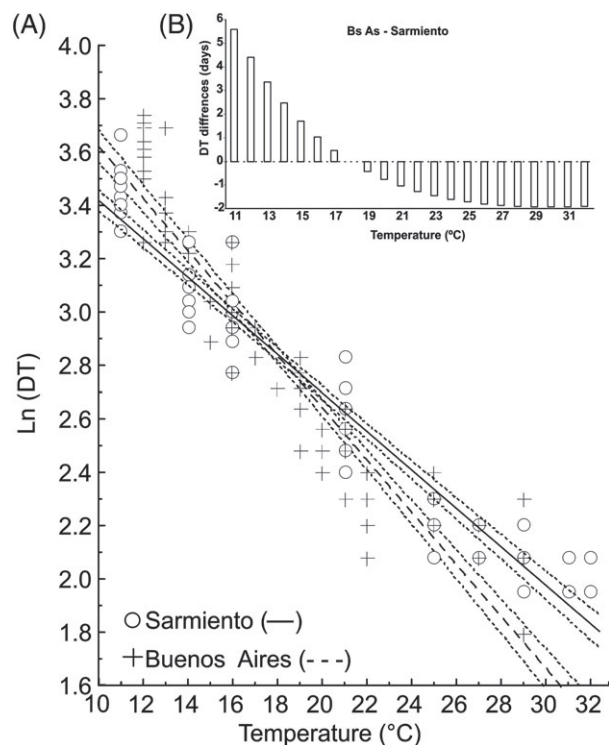


Fig. 2. (A) Natural logarithm of the development time of *Ochlerotatus albifasciatus* as a function of rearing temperature for Sarmiento and Buenos Aires. (B) Differences between populations with respect to the development time ($y = \text{TD Buenos Aires} - \text{TD Sarmiento}$) estimated from the linear fit equation.

$c = -0.0540$, $r^2 = 0.90$) and Sarmiento Valley ($m = 0.0049$, $c = -0.0225$, $r^2 = 0.89$) (figure not shown). The development threshold ($-c/m$) estimated for the population of Sarmiento valley (4.59 ± 3.08 °C) was lower than that of the population of Buenos Aires (8.06 ± 1.81 °C). The thermal constant ($1/m$) was higher for the population of Sarmiento Valley (204.08 ± 7.83 degree days) than for the population of Buenos Aires (149.25 ± 2.6 degree days).

Total survival (instar I-Pupa) of the individuals from temperate climate (Buenos Aires) was similar across the range of thermal conditions (0.15–0.18). For the arid climate (Sarmiento Valley), survival was significantly lower at hot temperatures (0.16) than at warm (0.50) and cold temperatures (0.56) (Table 2).

The instar I showed different survival patterns in each region (Table 2). In Buenos Aires, the survival of instar I increased significantly with temperature, whereas in the Sarmiento Valley, the survival of instar I was significantly lower at hot temperatures than at warm and cold temperatures. The survival of instar III of Buenos Aires and that of instar III and IV of Sarmiento were significantly higher at cold temperatures.

The length of the wing (the estimator of the size of adults) showed an inverse linear relationship with temperature both for Buenos Aires ($r^2 = 0.78$; $P < 0.0001$, $n = 97$) and for Sarmiento ($r^2 = 0.79$; $P < 0.0001$, $n = 99$) (Fig. 3). In both populations, the wing length of individuals ranged from 2.8 to 4.5 mm. The confidence intervals (CIs) of both slopes ($m_{\text{Sar}} = -0.05$; $m_{\text{BsAs}} = -0.06$) were overlapped, suggesting that there would be non-significant differences between the rate of change in the size as a function of temperature (test for parallelism: $P = 0.22$). The CIs of the ordinate at the origin of both functions ($c_{\text{Sar}} = 4.69 \pm 0.13$; $c_{\text{BsAs}} = 4.91 \pm 0.16$) were overlapped.

Discussion

The inverse relationship between temperature and the time of development in both populations was expected for insects. However, comparatively individuals of the Patagonian population (Sarmiento) appear to have different patterns of responses according to local thermal characteristics, cold temperatures, whereas the populations of a temperate climate (Buenos Aires) according to warmer temperatures. Such differences may result from possible optimizations in response to regional thermal conditions. In the population from Sarmiento Valley, this was reflected in shorter development times and higher survival rates at cold temperatures, and lower development thresholds whereas in the Buenos Aires population in a shorter development times at warm temperatures and higher thresholds of development, but not in survival.

Although the success of the population will depend very heavily on the survival of each stage, a shorter development time could ensure an important stage of the life cycle. For example, completing the development before the puddle drying is very important in survival respect (Fischer *et al.*, 2002). The individuals from Sarmiento performed better at cold temperatures because they took less time to develop and survived more than those from Buenos Aires. Similar characteristics have been observed in *Aedes impiger* (Diptera: Culicidae) Walker 1848

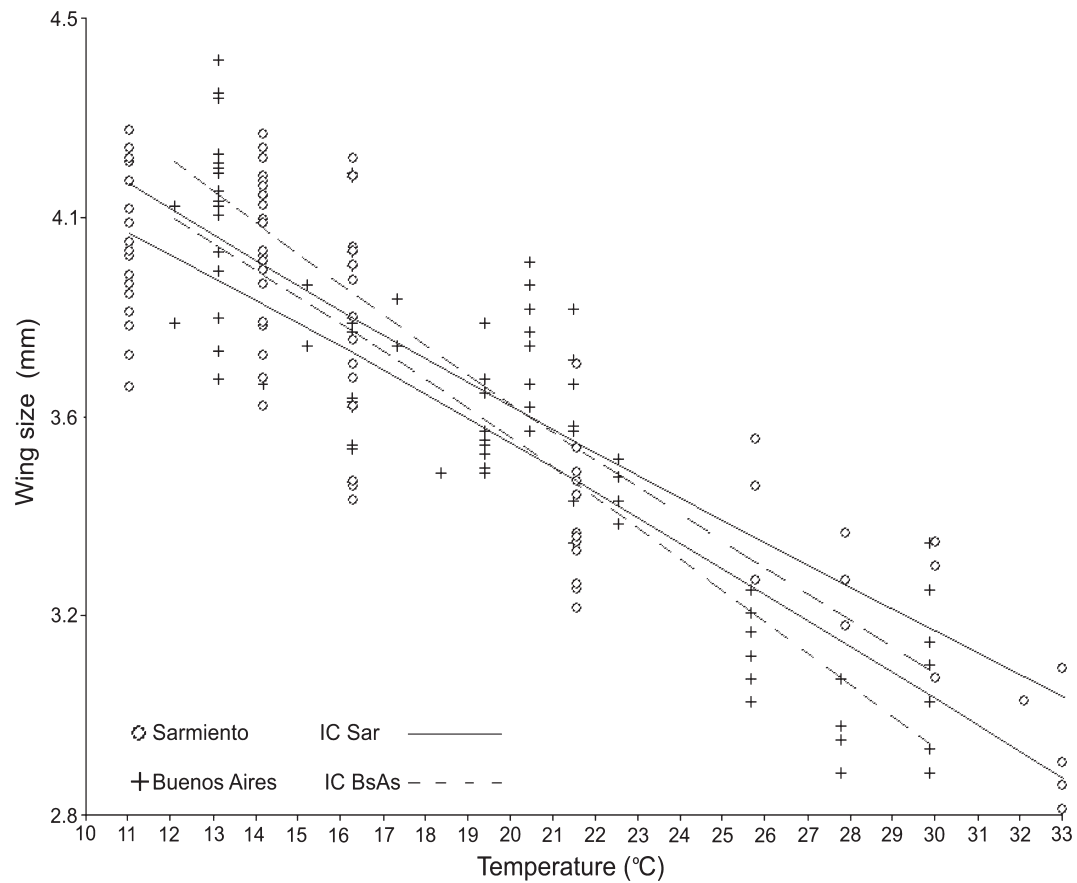


Fig. 3. *Ochlerotatus albifasciatus* wing size as a function of temperature of rearing immature for Sarmiento (Cold) and Buenos Aires (Temperate). The linear fit line is in the middle of the Interval of confidence of Sarmiento (IC Sar) and Buenos Aires (IC Bs AS) (not shown).

Table 2. Survival by instar and the total under different thermal ranges for Buenos Aires and Sarmiento.

| Instar | Buenos Aires | | | Sarmiento | | |
|--------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | 11–16 °C | 17–22 °C | 25–32 °C | 11–16 °C | 17–22 °C | 25–32 °C |
| I | 0.70 ^a (299) | 0.81 ^b (215) | 0.97 ^c (163) | 0.99 ^A (120) | 0.95 ^A (40) | 0.57 ^B (140) |
| II | 0.79 ^a (209) | 0.79 ^a (174) | 0.81 ^a (158) | 0.88 ^A (119) | 0.95 ^A (38) | 0.85 ^A (80) |
| III | 0.61 ^a (165) | 0.54 ^{ab} (138) | 0.47 ^b (128) | 0.90 ^A (105) | 0.86 ^{AB} (36) | 0.68 ^B (68) |
| IV | 0.58 ^a (101) | 0.59 ^a (75) | 0.55 ^a (60) | 0.85 ^A (95) | 0.77 ^{AB} (31) | 0.67 ^B (46) |
| P | 0.82 ^a (59) | 0.83 ^a (44) | 0.73 ^a (33) | 0.83 ^A (81) | 0.83 ^A (24) | 0.70 ^A (31) |
| Total | 0.15 ^a (299) | 0.18 ^a (215) | 0.15 ^a (163) | 0.56 ^A (120) | 0.50 ^A (40) | 0.16 ^B (140) |

The sample size is indicated in brackets.

Different superscript letters indicate significant differences between thermal ranges at each instar.

and *Ae. nigripes* (Diptera: Culicidae) Zetterstedt, 1838 from cold desert regions (Danks *et al.*, 1994).

Ochlerotatus albifasciatus is one of the few floodwater mosquito species adapted to the southern extreme of South America. Lane (1953) suggested a possible Patagonic region among seven centres of endemism and dispersal for the Culicidae in the Neotropical region. Under this concept, some features in both populations studied would be conserved, such as resistance to freezing eggs (Garzón *et al.*, 2013). However, other traits of the life history show patterns that adjust to the different local conditions such as an increase in larval survival

and a faster development at low temperatures. These plasticities of a response to particular thermal conditions could be a characteristic of the species that explains its wide geographical distribution from temperate latitudes to colder latitudes, as the Pampas and Patagonian region, respectively. However, its limited distribution northward suggests that there are other limiting factors or conditions (Begon *et al.*, 2006) besides temperature (e.g. food, interspecific competition, parasitism and predation).

Unlike that observed for other species (Trimble & Smith, 1978), the development time for each instar showed variable patterns regarding total development time (faster in Sarmiento

Valley than in Buenos Aires at cold temperatures and vice versa). By contrast, the greatest differences between development times at low temperatures could be because the instars that most contribute to the differences (instar IV and pupae) last longer. We cannot explain why the second-instar larvae of temperate regions developed more quickly than those from cold regions at cold temperatures.

With respect to the development parameters, the thermal constant obtained for the population of Buenos Aires required fewer degree days to complete larval development, indicating a reduction in the time of emergence of adults with respect to the population of the Sarmiento Valley. This could be related to the short duration of the small ponds and puddles formed from rainwater during the summer season in Buenos Aires. In the northern hemisphere, Trudgill & Perry (1994) showed that species from higher geographical latitudes with a low threshold of development and adapted to cold climate develop faster at low temperatures, whereas species from lower latitudes with higher thresholds develop faster at warmer temperatures.

In *Oc. albifasciatus* as in most multivoltine poikilotherms, distributed along a latitudinal gradient, the change in the parameters of the life cycle would contribute to cope with the changes in the length of the favourable season (Roff, 1980) i.e. a decrease in the development time and threshold with a decreasing favourable season by increasing latitudes. These geographical variations would obey a rule of 'greater latitude, lower development threshold and greater thermal constant' demonstrated at an interspecific level (Honek, 1996) and even, in some cases, at an intraspecific level. The results of the present study suggest that the *Oc. albifasciatus* populations studied would constitute another example of this last level.

Under the assumption that the temperature range studied represents the spring-summer period for the arid region (Sarmiento Valley) and the annual temperature range for the temperate region (Buenos Aires), and without other mortality factors, the results suggest that under field conditions the cold temperatures could be more favourable for survival of individuals in Sarmiento, e.g. at the beginning or end of the summer. While in Buenos Aires the survival, although low, would not show a favourable temperature range for survival during the year. The higher survival at low temperatures of the individuals from Sarmiento Valley would optimize the proliferation of *Oc. albifasciatus*.

The key to understanding the patterns of overall survival seem to be at the stage level. For example, we observed increased survival of the first-, third- and fourth-instar larvae of the population of Sarmiento Valley at low temperatures and an inverse pattern for the first-instar larvae of the population of Buenos Aires. By contrast, some instars were not affected by the thermal conditions, suggesting that different instars of development have different responses to the breeding temperatures. The survival patterns of the individuals of *Oc. albifasciatus* of the different populations studied suggest a plasticity of response to thermal conditions according to the region of origin, in agreement with that observed among strains of *Ae. aegypti* from different regions of the United States (Tun-Lin *et al.*, 2000).

It can be concluded that comparatively the best temperature for survival and faster development of the population from Sarmiento is within a cold temperature range. Moreover, for the

population from Buenos Aires comparatively only the development showed a better performance in a warm temperature range.

Acknowledgements

To Dr Oscar Jensen for logistical support in the valley of Sarmiento during the sampling period.

References

- Avilés, G., Sabattini, M.S. & Mitchell, C.J. (1992) Transmission of western equine encephalomyelitis virus by Argentine *Aedes albifasciatus* (Diptera: Culicidae). *Journal of Medical Entomology*, **29**, 850–853.
- Bachmann, A.O. & Bejarano, J.E.R. (1960) Dispersión de mosquitos en la Patagonia (Dipt. Culicidae-Culicinae). *Neotropica*, **6**, 70–71.
- Begon, M., Townsend, C.R. & Harper, J.L. (2006) *Ecology: From Individuals to Ecosystems*, 4th edn. Blackwell Publishing, Manden, ND.
- Campos, R.E. (2008) Long-term storage and viability of *Ochlerotatus albifasciatus* egg (Diptera: Culicidae). *Memórias do Instituto Oswaldo Cruz*, **103**, 115–117.
- Campos, R.E. & Sy, V.E. (2006) Variation in the hatching response of *Ochlerotatus albifasciatus* egg batches (Diptera: Culicidae) in the temperate Argentina. *Memórias do Instituto Oswaldo Cruz*, **101**, 47–53.
- Chatterjee, S., Hadi, A.S. & Price, B. (2000) *Regression Analysis by Example*, 3rd edn. John Wiley & Sons, Inc., New York, NY.
- Clements, A.N. (1992) *The Biology of Mosquitoes, Vol. I. Development, Nutrition and Reproduction*. Chapman & Hall, London.
- Danks, H.V., Kukal, O. & Ring, R.A. (1994) Insect cold-hardiness: insights from the Arctic. *Arctic*, **47**, 391–404.
- Fava, F.D., Ludueña Almeida, F.F., Almirón, W.R. & Brewer, M. (2001) Winter biology of *Aedes albifasciatus* (Diptera: Culicidae) from Córdoba, Argentina. *Journal of Medical Entomology*, **36**, 253–259.
- Fischer, S., Marinone, M.C. & Schweigmann, N. (2002) *Ochlerotatus albifasciatus* in rain pools of Buenos Aires: seasonal dynamics and relation to environmental variables. *Memórias do Instituto Oswaldo Cruz*, **97**, 767–773.
- Fleiss, J.L. (1981) *Statistical Methods for Rates and Proportions*. John Wiley and Sons, New York, NY.
- Garzón, M.J., Jensen, O. & Schweigmann, N. (2013) Resistance to freezing temperatures in *Aedes (Ochlerotatus) albifasciatus* (Macquart) eggs (Diptera: Culicidae) from two different climatic regions of Argentina. *Journal of Vector Ecology*, **38**, 339–344.
- Gerberg, E.J., Barnard, D.R. & Ward, R.A. (1994) *Manual for Mosquito Rearing and Experimental Techniques*. American Mosquito Control Association, Inc., Lake Charles, Louisiana.
- Honek, A. (1996) Geographical variation in thermal requirements for insect development. *European Journal of Entomology*, **93**, 303–312.
- Honek, A. (1999) Constraints on thermal requirements for insect development. *Entomological Science*, **2**, 615–621.
- Lane, J. (1953) *Neotropical Culicidae*, Vol. 1 and 2. University of Sao Paulo, Sao Paulo.
- Leica Application Suite V3.7 (2010) *LAS User Manual 2010*. Leica Microsystems (Switzerland) Ltd., Heerbrugg, Switzerland.
- Loetti, M.V., Burroni, N.E., Schweigmann, N. & de Garin, A. (2007) Effect of different thermal conditions on the pre-imaginal biology of *Culex apicinus* (Philipi, 1865) (Diptera: Culicidae). *Journal of Vector Ecology*, **32**, 106–111.

- Peel, M.C., Finlayson, B.L. & McMahon, T.A. (2007) Updated world map of the Koppen-Geiger climate classification. *Hydrology and Earth System Science*, **4**, 439–473.
- Prosen, A.F., Carcavallo, R.V. & Martínez, A. (1962) Culicidae de Bolivia (Diptera). *Anales del Instituto de Medicina Regional*, **6**, 59–124.
- Roff, D. (1980) Optimizing development time in a seasonal environment: the “ups and downs” of clinal variation. *Oecologia*, **45**, 202–208.
- Schäfer, M.L. & Lundström, M.O. (2006) Different responses of two floodwater mosquito species, *Aedes vexans* and *Ochlerotatus sticticus* (Diptera: Culicidae), to larval habitat. *Journal of Vector Ecology*, **31**, 123–128.
- Sweeney, B.W. & Vannote, R.L. (1981) *Ephemerella* mayflies of White Clay Creek: bioenergetic and ecological relationships among six coexisting species. *Ecology*, **62**, 1353–1369.
- Teng, H.J. & Apperson, C.S. (2000) Development and survival of immature *Aedes albopictus* and *Aedes triseriatus* in the laboratory: effects of density, food, and competition on response to temperature. *Journal of Medical Entomology*, **37**, 40–52.
- Trimble, R.M. & Smith, S.M. (1978) Geographic variation in development time and predation in tree-hole mosquito, *Toxorhynchites rutilus septentrionalis* (Diptera: Culicidae). *Canadian Journal of Zoology*, **56**, 2156–2165.
- Trudgill, D.L. & Perry, J.N. (1994) Thermal time and ecological strategies- a unifying hypothesis. *Annals of Applied Biology*, **125**, 521–532.
- Tun-Lin, W., Burkot, T.R. & Kay, B.H. (2000) Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. *Medical and Veterinary Entomology*, **14**, 31–37.
- Williams, D.D. & Felmate, B.W. (1992) *Aquatic Insects*. CAB International, Wallingford, U.K.
- Wise, E.J. (1980) Seasonal distribution and life histories of Ephemeroptera in a Northumbrian River. *Freshwater Biology*, **10**, 101–111.
- Zar, J.H. (1996) *Biostatistical Analysis*. Prentice-Hall, Upper Saddle River, NJ.

Accepted 14 March 2015